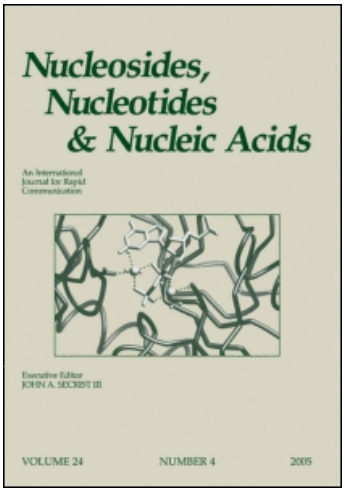

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Oligonucleotide Synthesis Using Ionic Liquids as Soluble Supports

Robert A. Donga[a]; Matthew Hassler[a]; Tak-Hang Chan[a]; Masad J. Damha[a]
[a] Department of Chemistry, McGill University, Montreal, Canada

To cite this Article Donga, Robert A. , Hassler, Matthew , Chan, Tak-Hang and Damha, Masad J.(2007) 'Oligonucleotide Synthesis Using Ionic Liquids as Soluble Supports', Nucleosides, Nucleotides and Nucleic Acids, 26: 10, 1287 — 1293
To link to this Article: DOI: 10.1080/15257770701530533
URL: http://dx.doi.org/10.1080/15257770701530533

Nucleosides, Nucleotides, and Nucleic Acids, 26:1287–1293, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701530533

Taylor & Francis
Taylor & Francis Group

OLIGONUCLEOTIDE SYNTHESIS USING IONIC LIQUIDS AS SOLUBLE SUPPORTS

Robert A. Donga, Matthew Hassler, Tak-Hang Chan, and Masad J. Damha □
Department of Chemistry, McGill University, Montreal, Canada

□ *The continuing evolution of the methodology for the solution-phase synthesis of oligonucleotides using soluble ionic tags as handles for easy purification is described. This methodology may provide a more cost efficient route for the large scale synthesis of oligonucleotides.*

Keywords Oligonucleotides; soluble ionic tags

INTRODUCTION

In recent years oligonucleotides have seen widespread use in the development of therapeutics and diagnostic applications, including chip-based DNA microarrays,[1,2] though the number of approved therapeutics thus far has been small. Traditional solid-phase approaches to synthesis have been sufficient to supply need up until now. While the use of solid supports have been successful for these smaller scale applications,[3] they have all the problems generally associated with heterogeneous reaction conditions. In addition, the supports and the excess amidite reagents (see Figure 1)[4] can constitute up to 60% of the total materials cost, making this type of synthesis very expensive. With a greater number of potential oligonucleotide based drugs reaching late phase clinical trials, development of a more cost efficient method is becoming highly desirable.

The use of a fluorous phase technique for organic synthesis has been advocated in recent years[5–9] and has been demonstrated for small molecules and some biopolymers but the technique could be limited for large scale applications by the expense of perfluoroalkane solvents, the need for specialized fluorinated reagents, and the energy cost associated with the temperature switch.

We would like to thank the National Science and Engineering Council of Canada (NSERC) for funding of our research.

Address correspondence to Masad J. Damha, Department of Chemistry, McGill University, Montreal H3A 2K6, Canada. E-mail: masad.damha@mcgill.ca

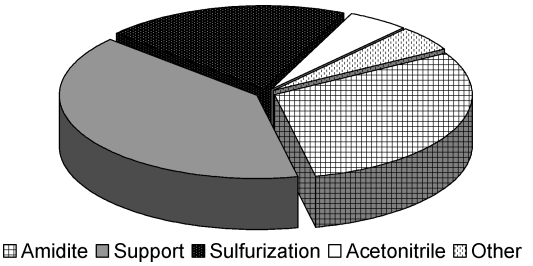

FIGURE 1 Proportion of cost by component (2000).

Soluble polymer supports have been explored as a possible improvement to solid-phase techniques since soluble-supported syntheses retain many of the advantages of conventional solution-phase chemistry, while still permitting facile purification of the product. Thus Bonara et al.[10] have shown that soluble polyethylene glycol (PEG) may be used successfully in the synthesis of oligonucleotides. Some limitations of using soluble polymer supports include a low capacity of loading, limited solubility during the synthesis of longer sequences, and often low aqueous solubility[11,12] as well as, in the case of oligonucleotide synthesis, an energy intensive cooling required for purification.

Recently, there has been considerable interest in the use of ionic liquids as "green" alternatives to more traditional reaction media. We have previously demonstrated[13] that an ionic liquid supported synthesis (ILSS) technique can be successfully applied to oligonucleotide synthesis using an imidazolium-based tetrafluoroborate ionic tag, using simple precipitation and phase separation methods without the need for chromatography to obtain reasonably high product purity at each step. Herein we explore the optimization of the technique as it is moved to the gram scale and investigate other ionic moieties that may be used.

MATERIALS AND METHODS

Synthesis of Ionic Support (Tag)

The ionic supports are simply generated from amines or phosphines, such as triethylamine, 1-methylimidazole or tributylphosphine, mixed in

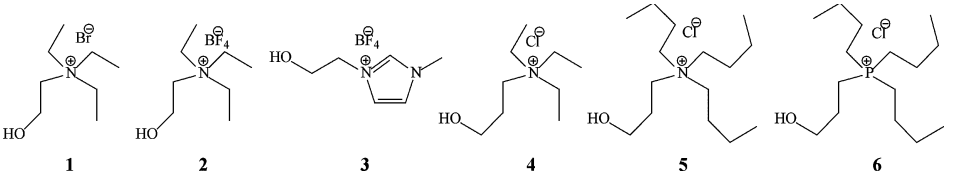

FIGURE 2 Structures of ionic tags.

equimolar amounts with aliphatic halo-alcohols, such as 2-bromoethanol or 2-chloropropanol, and refluxing in a suitable solvent such as ethanol or, in some cases, using microwave activation in solvent free conditions.[14] Exchange of the anion is simply achieved by dissolving or suspending this material in acetone and adding an excess of the desired counter-ion, such as tetrafluoroborate, generally as a sodium salt, stirring for 12 hours and filtering away the remaining insoluble solid. The desired material is recovered by removing the solvent under vacuum (Table 1).

Linking Ionic Tag to Nucleoside

The desired ionic tag is dissolved in acetonitrile and mixed with a limiting amount of 5′-*O*-dimethoxytrityl-3′-*O*-succinyl-thymidine ($^{DMT}T_{Succ}$) in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). The reactions are allowed to continue for 3–7 days. The desired products are generally recovered by precipitation from ether followed by a water-dichloromethane extraction, isolating the desired product from the organic layer (Table 1).

Detritylation of First Nucleoside ($^{HO}T_{Succ\text{-}IL}$)

To $^{DMT}T_{Succ\text{-}IL}$, where the IL subscript can indicate any of the ionic tags that have been prepared (see Table 1), is added any of a variety of detritylating reagents, such as 3% v/v trifluoroacetic acid (TFA) in dichloromethane or 1M zinc bromide ($ZnBr_2$) in 85:15 v/v dichloromethane/2-propanol, with or without approximately 1 mol equivalent of pyrrole. The product of the reaction is generally precipitated from 10% ethyl acetate/diethyl ether and filtered. The precipitate is rinsed with 10% ethyl acetate/diethyl ether, and is recovered from the filter by dissolving in acetonitrile and evaporating the solvent under reduced pressure. This is repeated (normally 3–5 times for $ZnBr_2$, 10–15 times for TFA) until no trityl is detectable by TLC and/or ESI-MS.

Coupling of Nucleotides ($^{DMT}NpT_{Succ\text{-}IL}$ (N = dA, dC, dG, dT))

$^{HO}T_{succ\text{-}IL}$ is dissolved in anhydrous acetonitrile and mixed, in the presence of 2,3-dicyanoimidazole (DCI), with 2 molar equivalents of a desired nucleoside 3′-*O*-phosphoramidite, $^{DMT}N_P$, where N may be dA, dC, dG, or dT. The reaction is allowed to progress for 45 minutes whereupon an excess of a simple alcohol is added and allowed to quench the excess amidite for 15 minutes. The intermediate product is then precipitated several times from 10% v/v ethyl acetate in diethyl ether. The purified intermediate is then capped using acetic anhydride, catalyzed with DMAP over a 10-minute period, followed by an oxidation step with *tert*-butyl hydroperoxide in

TABLE 1 Yield and characterization

Compound	Scale (mmol)	Yield (g)	% Yield	ESI-MS (M^a, m/z) Calculated	Observed
2-Hydroxyethyl-triethylammonium bromide	60	13.52	93%	146.3	146.4
2-Hydroxyethyl-triethylammonium tetrafluoroborate	30	6.40	88%	146.2	146.0
3-(2′-Hydroxyethyl)-1-methylimidazolium tetrafluoroborate	50	7.20	72%	127.1	127.1
3-Hydroxypropyl-triethylammonium chloride	30	2.89	96%	243.8	243.7
3-Hydroxypropyl-tributylammonium chloride	10	5.12	88%	160.2	160.1
3-Hydroxypropyl-tributylphosphonium chloride	10	3.81	87%	261.2	261.1
$^{DMT}T_{Succ\text{-}hydrxyethylmethylimidazolium\ tetrafluoroborate}$	7	5.21	88%	753.3	753.3
$^{DMT}dG_pT_{Succ\text{-}hydrxyethylmethylimidazolium\ tetrafluoroborate}$	2	2.40*	100%*	1205.2	1205.4
$^{DMT}dC_pdG_pT_{Succ\text{-}hydrxyethylmethylimidazolium\ tetrafluoroborate}$	1	1.76*	100%*	1651.3	1651.5
$^{DMT}T_{Succ\text{-}2\text{-}ethoxytriethylammonium\ tetrafluoroborate}$	0.4	0.26	70%	772.4	772.3
$^{DMT}T_{Succ\text{-}3\text{-}propoxytributylphosphonium\ chloride}$	0.5	0.10	22%	887.5	887.3

[a]From impure product yield due to lack of observable product loss in purification media and lack of starting material in final product by MS.

decane, lasting 10-minutes. The final product is again purified by precipitation. Detritylation is achieved as described previously and the entire process is repeated until an oligomer of desired length and composition is completed.

RESULTS AND DISCUSSION

In our earlier work,[13] we identified that the detritylation step was a major issue for the proposed methodology. Our subsequent work at larger scale has shown that the difficulty is limited to the first nucleoside attached to the ionic moiety through the linker and that subsequent nucleotides detritylate in a manner similar to that of the free nucleoside. The impediment to detritylation of the first nucleoside appears to be due to the close proximity of the ionic tag to the site at which protonation must occur (5′-O) to initiate trityl cleavage. As the oligomer grows in length the site of protonation is spatially displaced from the positive charge of the ionic tag, allowing the rate of cleavage to revert to the more acceptable level normally observed for free nucleosides and solid supported oligomers. A potential method to overcome the slow detritylation of the first nucleoside is to use a Lewis acid such as zinc bromide instead of a Brønsted acid to initiate the deprotection since weaker charge-charge repulsion would be expected between the ion tag and the zinc coordinated 5′-oxygen. In practice, the improvement is minor when $ZnBr_2$ is used alone. When the Lewis acid is used in conjunction with a trityl scavenger such as pyrrole,[15] however, a significant improvement is observed. Whereas upwards of twelve treatments with Brønsted acid are required for complete detritylation, complete deprotection is achieved in three treatments using zinc bromide and pyrrole. There are several drawbacks of this procedure, including the formation of an insoluble brown precipitate that entrains some of the desired product. In addition, Kierzek et al.[16] have shown that deacylation of the protected bases can occur when zinc bromide is used to detritylate in the presence of methanol, which is frequently used as a solvent or co-solvent and deacylation of both guanine and cytidine have been observed experimentally. Finally, the high level of toxicity associated with pyrrole makes this reagent undesirable for large scale applications. These factors have led us to revert to the use of TFA for detritylation even though many treatments are required for the first nucleoside. An alternate protecting scheme for the first nucleoside could be introduced while the traditional scheme is maintained for the growth of the oligomer.

In order to successfully separate the coupled oligomer from the monomer at each coupling step of the synthesis cycle it is necessary to quench the excess amidite with an alcohol prior to oxidation of the phosphite triester. This enhances the amidites solubility in the precipitation

medium, generally 10% v/v ethyl acetate in ethyl ether, allowing its removal. Initially methanol and ethanol were used to quench the excess amidite but it was found that even over short periods of time they can transesterify the phosphite triester yielding undesirable compounds. Isopropanol was found to cause transesterification at a much slower rate than methanol or ethanol, while no transesterification was observed over after a ten hour exposure with *tert*-butanol.

The reported methodology[13] employs iodine/water as the oxidant, requiring an extraction step for purification. Extractions with these products generally are found to be difficult because they have the capacity to behave as strong emulsifiers and the emulsions they form are very slow to break. Other nonaqueous based oxidants have been demonstrated to be effective in oligonucleotide synthesis such as *tert*-butyl hydroperoxide.[17] Used as a 6–7 M solution in decane in 10 mole equivalents, it was found that the oxidation proceeded very rapidly (<5 minutes).

Syntheses of the ammonium based ionic tags (Figure 1) are in the preliminary stages of investigation. Thus far they have been found to form in satisfactorily high yields but the derivatization reaction to attach the tags to the first nucleoside has been problematic. This is more likely due to the small scale of the reactions that have been performed and the higher hydroscopicity of the ammonium ionic tags since water is a competitor to the nucleoside in this reaction. The success of the imidazolium based tag gives us a high level of confidence that these alternative tags can be tuned to function as desired.

REFERENCES

1. Fodor, S.P.; Rava, R.P.; Huang, X.C.; Pease, A.C.; Holmes, C.P.; Adams, C.L. Multiplexed biochemical assays with biological chips. *Nature* **1993**, 364, 555–556.
2. Barone, A.D.; Beecher, J.E.; Bury, P.A.; Chen, C.; Doede, T.; Fidanza, J.A.; McGall, G.H. Photolithographic synthesis of high-density oligonucleotide probe arrays. *Nucleosides, Nucleotides Nucleic Acids* **2001**, 20, 525–531.
3. Damha, M.J.; Ogilvie, K.K. Oligoribonucleotide synthesis. The silyl-phosphoramidite method. *Methods in Molecular Biology* **1993**, 20, 81–114.
4. a) Pon, R.T.; Yu, S.; Guo, Z.; Deshmukh, R.; Sanghvi, Y.S. Reusable solid-phase supports for oligonucleotides and antisense therapeutics. *J. Chem. Soc. Perkin Trans.* **2001**, 1, 2638–2643; b) Sanghvi, Y.S. personal communication.
5. Horvath, I.T.; Rabai, J. Facile catalyst separation without water: Fluorous biphase hydroformylation of olefins. *Science* **1994**, 266, 72–75.
6. Studer, A.; Hadida, S.; Ferritto, R.; Kim, S.-Y.; Jeger, P., Wipf, P.; Curran, D.P. Fluorous synthesis: A fluorous-phase strategy for improving separation efficiency in organic synthesis. *Science* **1997**, 275, 823–826.
7. Horvath, I.T. Fluorous biphase chemistry. *Accounts Chem. Res.* **1998**, 31, 641–650.
8. Betzemeier, B.; Knochel, P. Palladium-catalyzed cross-coupling of organozinc bromides with aryl iodides in perfluorinated solvents. *Angewandte Chemie* **1997**, 36, 2623–2624.
9. Wende, M.; Meier, R.; Gladysz, J.A. Fluorous catalysis without fluorous solvents: A friendlier catalyst recovery/recycling protocol based upon thermomorphic properties and liquid/solid phase separation. *J. Am. Chem. Soc.* **2001**, 123, 11490–11491.

10. Bonora, G.M.; Scremin, C.L.; Colonna, F.P.; Garbesi, A. HELP (high efficiency liquid phase) new oligonucleotide synthesis on soluble polymeric support. *Nucleic Acids Res.* **1990**, 18, 3155–3159.
11. Gravert, D.J.; Janda, K.D. Organic synthesis on soluble polymer supports: Liquid-phase methodologies. *Chem. Rev.* **1997**, 97, 489–509.
12. Toy, P.H.; Janda, K.D. Soluble polymer-supported organic synthesis. *Accounts Chem. Res.* **2000**, 33, 546–554.
13. Donga, R.A.; Khaliq-Uz-Zaman, S.M.; Chan, T.-H.; Damha, M.J. A novel approach to oligonucleotide synthesis using an imidazolium ion tag as a soluble support. *J. Org. Chem.* **2006**, 71, 7907–7910.
14. Law, M.C.; Wong, K.-Y.; Chan, T.H. Solvent-free route to ionic liquid precursors using a water-moderated microwave process. *Green Chemistry* **2002**, 4, 328–330.
15. Reese, C.B.; Serafinowska, H.T.; Zappia, G. An acetal group suitable for the protection of 2′-hydroxy functions in rapid oligoribonucleotide synthesis. *Tetrahedron Lett.* **1986**, 27, 2291–2294.
16. Kierzek, R.; Ito, H.; Bhatt, R.; Itakura, K. Selective N-deacylation of N,O-protected nucleosides by zinc bromide. *Tetrahedron Lett.* **1981**, 22, 3761–3764.
17. Hayakawa, Y.; Uchiyama, M.; Noyori, R. Nonaqueous oxidation of nucleoside phosphites to phosphates. *Tetrahedron Lett.* **1986**, 27, 4191–4194.